REMARKS

The Examiner objected to the Specification for lack of a Cross-Reference to Related applications. Applicants herein amend the specification to include a proper cross-reference, and now submit that this objection is traversed.

Following entry of the present amendment, new claims 27-36 remain in the application for consideration. Claims 1-26 are herein cancelled without prejudice, including claims 2-7, 15-16, and 20-26 which were withdrawn from consideration. No new matter is added.

Rejections under 35 USC §101

Claim 8 was rejected under 35 USC §101 because the claimed recitation of a use without setting forth process steps results in an improper claim under the Patent Laws.

Applicants herein cancel claim 8 without prejudice and submit that this rejection is now moot.

Rejections under 35 USC §112, First Paragraph

Claims 1, 8-14, and 17-19 were rejected as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors had possession of the

invention at the time the application was filed. Applicants respectfully traverse the rejection.

To address the rejection, Applicants submit herewith new claims 27-36. Independent Claim 27 recites as follows:

> 27. A method of affinity separation, comprising the steps of:
>
> (a) providing a sample containing a target analyte;
>
> (b) providing a matrix comprising an immobilized protein, said immobilized protein comprising one or more modifications that (i) increase the stability of said protein in alkaline conditions, and (ii) permit said protein to bind to said target analyte, said one or more modifications selected from the group consisting of:
>
> (1) deleting one or more Asn residues in said protein;
>
> (2) substituting one or more Asn residues in said protein for an amino acid that is less sensitive to alkaline conditions;
>
> (3) chemically modifying one or more Asn residues in said protein; and
>
> (4) combinations thereof;
>
> (c) contacting said sample and said matrix, wherein said target analyte binds to said immobilized protein; and
>
> (d) isolating said target analyte from said matrix.

As recited in Claim 27, the present invention is directed to a method of affinity separation that utilizes a novel matrix that contains an immobilized protein that has been modified to

be resistant to the effects of alkaline pH. In step (b) of claim 27, a matrix is provided that comprises an immobilized protein, wherein the immobilized protein comprises one or more modifications that (i) increase the stability of the protein in alkaline conditions, and (ii) permit the protein to bind to a target analyte. These limitations are disclosed in the specification at page 7, lines 30-37, and continuing to page 8, lines 1-4.

Step (b) of claim 27 recites additional structural limitations of the protein regarding the specific type of modifications. Specifically, the claimed modifications are selected from (1) deletion of one or more Asn residues in the protein; (2) substitution of one or more Asn residues for an amino acid that is less sensitive to alkaline conditions; (3) chemical modification of one or more Asn residues; and (4) combinations thereof. These limitations are disclosed in the specification at page 4, lines 26-33. Together, these limitations explicitly recite structural and functional features common to all members of the genus.

In the rejection, the Examiner indicated that the claims encompassed a broad genus and do not provide guidance as to what structural features all of these proteins share that will allow them to continue to function as a ligand and will also allow them to resist alkaline conditions. On page 7 of the office

action, the Examiner also indicated that the disclosure fails to describe the common attributes or characteristics that identify all of the members of the genus, or even a substantial portion thereof. Applicants submit that new claims 27-36 address these rejections.

Applicants submit that the limitations now recited in claim 27 are fully disclosed in the specification and are described in terms of detailed functional and structural characteristics, with a correlation between function and structure as required by the Written Description requirement as set forth in the Written Description Guidelines (66 FR 1099-1111 (January 5, 2001)). Claim 27 recites particular limitations that distinguish the claimed proteins in the matrix. For example, claim 27 recites modifications that increase the stability of the protein in alkaline conditions, and permit the protein to bind to the target analyte. Applicants point out that the modifications encompassed by Claim 27 do not result in a new binding affinity being achieved, but rather that the original affinity is not substantially impaired.

Claim 27 further recites that the modifications consist of deleting one or more Asn residues in the protein; substituting one or more Asn residues in the protein for an amino acid that is less sensitive to alkaline conditions; chemically modifying one or more Asn residues in the protein; or combinations

thereof. As indicated above, all these limitations are fully disclosed in the specification. Therefore, Applicants submit that claim 27 and the specification, as written, are sufficient to convey to an artisan skilled in the biochemical arts that the inventors had possession of the claimed invention at the time the application was filed.

On page 7 of the office action, the Examiner indicated that the art teaches that finding mutants that will retain their inherent binding characteristics and possess the increased stability that is required here cannot be taught (i.e., is inherently unpredictable). The Examiner cited Applicants' paper (Gulich et al., Protein Engineering 15:835-842 (2002)) as support for this conclusion, and particularly the passages reciting "These modifications may change the function or the potency of a protein or peptide" and "Different proteins are modified to a different extent since the deamidation/isomerization rate is highly sequence and conformation dependent". See Gulich et al., p. 835.

Applicants respectfully point out the Examiner is misreading the above passages and incorrectly concluding that finding particular mutant proteins is unpredictable. Applicants submit that the modifications referred to in the cited passage are those modifications that result from the alkaline sensitivity of asparagine residues (i.e., deamidation and

isomerization). These are the spontaneous chemical modifications that occur in alkaline sensitive amino acids (see first sentence of paragraph 2 in Gulich paper). The fact that such spontaneous chemical modifications change the protein function or potency is a major reason why there is a technical problem to be solved. Thus, this passage is not a discussion of the effects of mutations in the amino acid sequence to reduce the number of such sensitive asparagine residues in accordance with the method of the present invention. Rather, it is a description of the types of modifications that take place in an alkaline environment.

With regard to predictability, Applicants submit that the specification explicitly points out that modifications of the type recited in Claim 27 occur to residues that are exposed to alkaline conditions (see specification, page 7, line 30 and continuing to page 8, line 4). In one embodiment of the invention, it is preferably the surface residues that are modified, but not those residues involved in ligand binding. Additionally, it is known in the art that most Asn residues are found on the surface of proteins. Therefore, Applicants submit that the specification provides one skilled in the art with considerable guidance regarding which Asn residues to modify in accordance with the method of the present invention.

In sum, Applicants submit that the specification contains sufficient written description to inform a skilled artisan that the Applicants were in possession of the claimed invention as a whole at the time the application was filed. As shown above, Applicants disclose (and now claim) the method of the present invention by showing detailed relevant identifying characteristics of the immobilized protein portion of the claimed matrix, and in particular its functional characteristics coupled with known and disclosed correlation between structure and function. Applicants submit that an artisan skilled in biochemical affinity techniques and protein modifications would have understood that the Applicants were in possession of the claimed invention at the time of filing, and that this rejection is overcome.

Claims 1, 8-14, 17-19 were rejected because the specification, while being enabling for albumin-binding domain asparagine mutants, allegedly does not provide enablement for any asparagine "modified" proteinaceous ligand, and the specification allegedly does not enable any person skilled in the art to make and use the invention commensurate in scope with these claims. Applicants respectfully traverse the rejection.

At the outset, Applicants submit that newly presented claims 27-36 more concisely describe the claimed invention, and

in particular the matrix and protein used in the claimed method. In particular, claim 27 now recites positive method steps that clearly outline the claimed method of affinity separation. In addition, the matrix component recited in step (b) is believed to concisely describe the matrix in terms of structure and function as described above. The immobilized protein component of the matrix is recited to include one or more modifications that increase the stability of the protein under alkaline conditions, and permit the protein to bind to a target analyte by retaining substantially all of the native binding characteristics. The nature of the modifications are also specifically recited to be deletion of one or more Asn residues from the protein, substitution of one or more Asn residues for an amino acid residue that is less sensitive to alkaline conditions, chemical modification of one or more Asn residues, or a combination of these. Accordingly, the claims now define the invention with particularity and limit the recited proteins to a specific genus that meet the above combination of limitations.

The Examiner indicated that the level of predictability in the relevant art is unpredictable, and that the prior art shows that modifying proteins can have a destabilizing and unpredictable effect on the structure and function of the protein. However, as indicated above, Applicants submit that

the Examiner is misreading the passages quoted from Gulich et al. Applicants submit that the quoted passages are directed to a description of the types of modifications that take place in an alkaline environment, and not to the effects of mutations in accordance with the method of the present invention.

Applicants submit that the level of predictability in the relevant art is not unpredictable as asserted by the Examiner. On the contrary, Applicants disclose and claim that the Asn residues that participate in protein-analyte interactions should be avoided, and that it is well known that Asn residues are found primarily on the surface of proteins. Moreover, techniques are known in the biochemical arts that permit one of skill in the art to only modify those residues found on the exterior of the protein and exposed to solvent, for example from an examination of the three dimensional structure of the protein. Accordingly, Applicants submit that the level of predictability in the relevant art of protein modification is substantially predictable.

Applicants agree that the level of skill in the relevant art is high, likely the Ph.D. level. However, Applicants submit that this high level of skill of an ordinary skilled artisan would include a knowledge of protein structure and function, as well as knowledge of protein modification, including chemical and genetic approaches to such modifications. In addition, a

skilled artisan with this level of skill would appreciate the details of affinity separation methods, including affinity chromatography.

Accordingly, Applicants submit that the amount of direction provided in the specification is sufficient for one of ordinary skill in the art to make and use the invention. As mentioned above, the techniques to make and use the claimed invention are known in the art. For example, the techniques of affinity separation are well known in the biochemical arts. Affinity chromatography, in particular, is a common and well-established technique that is known to one of skill in the biochemical arts. Pages 1-2 and 4-5 of the specification describe in general terms the well known methods and techniques of affinity separations.

A skilled artisan would also have an understanding of protein structure and function, and could easily modify surface residues on a protein using well known chemical or genetic techniques (e.g., site directed mutagenesis or random mutagenesis as described generally in the specification). Applicants point out that the Written Description guidelines explicitly recite that what is conventional or well known to one of ordinary skill in the art need not be disclosed in detail. Accordingly, Applicants submit that the amount of direction and presence of working examples in the application is sufficient

for one of ordinary skill in the art to make and use the invention.

The Examiner also indicated that the quantity of experimentation needed to make and/or use the invention would be great. Applicants respectfully disagree. Applicants submit that the procedures described in the specification with respect to affinity separation methods and protein modifications are all well characterized and well known in the biochemical arts, and are well within the skill of the ordinary skilled artisan. Moreover, the choice of which Asn residues to modify and how the modifications are to be made are outlined in the specification, and techniques to accomplish these modifications are also well known in the art. Accordingly, Applicants submit that in light of these well known procedures, undue experimentation would not be necessary to make and use the present invention.

In light of the above arguments, Applicants submit that the application and claims is fully enabled and therefore that this rejection is overcome.

Rejections under 35 USC §112, Second Paragraph

Claims 1, 8-14 and 17-19 were rejected as being indefinite for failing to particularly point out and distinctly claim the invention. In view of claims 27-36 submitted herewith, Applicants submit that this rejection is moot.

Rejections under 35 USC §102

Claims 1, 8-14, and 17-19 were rejected as allegedly being anticipated by Ahern et al. (PNAS 84:675-679 (1987)). Applicants respectfully traverse the rejection.

Ahern et al. disclose site-directed mutagenesis of several asparagine residues in the enzyme triose phosphate isomerase (TIM) that result in increased thermostability of this enzyme at 100°C and pH 6. As disclosed by Ahern, the TIM enzyme functions as an affinity target, and an unmodified immunocomplex functions as the immobilized affinity ligand.

In contrast, claim 27 recites a method of affinity separation, comprising the steps of: (a) providing a sample containing a target analyte; (b) providing a matrix comprising an immobilized protein, the immobilized protein comprising one or more modifications that (i) increase the stability of the protein in alkaline conditions, and (ii) permit the protein to bind to the target analyte, the one or more modifications selected from the group consisting of: (1) deleting one or more Asn residues in the protein; (2) substituting one or more Asn residues in the protein for an amino acid that is less sensitive to alkaline conditions; (3) chemically modifying one or more Asn residues in the protein; and (4) combinations thereof; (c) contacting the sample and the matrix, wherein the target analyte

binds to the immobilized protein; and (d) isolating the target analyte from the matrix.

Applicants submit that Ahern et al. do not disclose or suggest the methods outlined in claim 27, much less the immobilized protein used in the matrix. Ahern et al. do not disclose or suggest an immobilized protein comprising one or more modifications that (i) increase the stability of the protein in alkaline conditions, and (ii) permit the protein to bind to the target analyte. Ahern et al. also do not disclose or suggest that such modifications may include deleting one or more Asn residues in the protein; substituting one or more Asn residues in the protein for an amino acid that is less sensitive to alkaline conditions; or chemically modifying one or more Asn residues in the protein. Significantly, Ahern discloses that the modified TIM enzyme functions as an affinity target, and an unmodified immunocomplex functions as the immobilized affinity ligand (i.e., the modified protein is a non-immobilized target). In contrast, the claimed invention requires a modified and immobilized affinity ligand. Accordingly, Applicants submit that Ahern et al. does not anticipate claim 27 or claims dependent therefrom, and that this rejection is overcome.

Applicants now submit that the claims are in condition for allowance, and respectfully request reconsideration and issuance of a timely Notice of Allowance.

If the Examiner has any questions or feels that a discussion with Applicants' representative would expedite prosecution, the Examiner is invited and encouraged to contact Applicants' undersigned representative at the telephone number listed below.

Respectfully submitted,

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